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INFLUENCE OF MYCORRHIZAL FUNGUS AND CERTAIN RHIZOBACTERIA ON ROOT-KNOT-NEMATODE (*MELOIDOGYNE INCOGNITA*) AND GROWTH OF BRINJAL (*SOLANUM MELONGENA* L)

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ABSTRACT

A 60 days greenhouse experiment was conducted to evaluate the efficacy of certain rhizobacteria (*P. fluorescens, B. subtilis, Azotobacter* spp.), Mycorrhizal fungi (*Glomus fasciculatum*) alone and in combination on the multiplying on *Meloidogyne incognita* and growth of brinjal. The experiment consisted of eighteen treatments with four replicates arranged in RBD. The plants treated with the combinations of certain rhizobacteria and Mycorrhizal fungus significantly suppressed number of galls per root system, second stage juveniles J_2 and improved plant growth over control, single treatments of rhizobacteria, Mycorrhizal fungusand Carbofuran 3G (chemical check). *P. fluorescens, B. subtilis, G. fasciculatum* when used in combination showed intermediary effects on both nematode reproduction and plant growth, while *Azotobacter* sp. was found to be least effective.

KEYWORDS: Brinjal, *Meloidogyne incognita*, *Pseudomonas fluorescens*, *Bacillus subtilis*, *Azotobacter* spp., *Glomus fasciculatum*, Carbofuran 3G

INTRODUCTION

Brinjal is an important vegetable crop grown in India. Root-knot nematode *Meloidogyne incognita* commonly infests this crop and causes yield reduction [3]. Root-knot nematodes (*Meloidogyne* spp.) are economically important plant pathogens, causing damage on many crops, mainly expressed as reductions of plant growth and lower yields. Traditionally, diseases caused by nematodes have been managed by nematicides and crop rotation [29]. Several strategies, including chemical nematicides, organic soil amendments, crop rotation, cover crops, resistant cultivars and biological control, have been developed for the management of plant parasitic nematodes [11]. While, environmental concerns and increased regulations have phased out the use of chemical fumigants [18], crop rotation and cover cropping are limited by scarcity of arable land. Evidence has been provided that integrating biological control using microbial antagonists with other feasible methods is amongst the most pragmatic strategies of managing the nematodes [17].

The rhizoplane and rhizosphere are colonized or otherwise occupied by many microorganisms and plant growth promoting rhizobacteria (PGPR) which are capable of providing substantial protection against nematode diseases [22]. PGPR especially belonging to the genera *Pseudomonas* and *Bacillus* have demonstrated potential for disease suppression without negative effects on the user, consumer or the environment [15].

Also induce systemic resistance against nematode pests [20]. *Pseudomonas flourescens* has induced systemic resistance and inhibited early root penetration of *Heterodera schachtii*, the cyst nematode in sugar beet [19, 20]. *P. fluorescens* has been investigated for its antagonistic effect to nematodes more extensively than others and found

effective against *Meloidogyne* spp. [7]. *Bacillus subtilis* can improve the plant growth by producing biologically active substances or by transforming unavailable mineral and organic compounds into available forms to plant [5]. Thus it may partially compensate the losses caused by plant parasitic nematodes and increase the crop yields. Arbuscular mycorrhizal (AM) fungi are soil-borne fungi that establish an obligate endophytic symbiosis with many plant species. When the hyphae come to inner cortex they become intercellular. Here the hyphae spreads in between cells and do not penetrate host cells. It is the site of exchange of nutrients between the host and the fungus. Essentially this transfer involves carbohydrates from plants to fungus and minerals especially phosphate from fungus to plants and have the potential of suppressing development of *Meloidogyne incognita*, *M. hapla* and their ability to induce galls [25]. *Glomus* species are the most diverse of the arbuscular mycorrhizal and are found in many soils all over the world. Interaction between AM fungi and N_2 fixing bacteria helper (N-fixing bacterium) has been proved to form a consortium benefiting the growth of a few plant species [28]. Therefore this study was planned with the objective to test the efficacy of rhizobacteria and Mycorrhizal fungus against *M. incognita* infection on brinjal.

MATERIALS AND METHODS

Nematode Inoculum

Meloidogyne incognita population, originally isolated from tomato was multiplied and maintained on susceptible eggplant in a greenhouse. Eggplants were uprooted carefully; roots were washed gently and cut in to small pieces. The roots were shaken vigorously for four minutes in a beaker containing 200 ml 1% NaOCl to release the eggs from egg-masses [9]. Eggs were collected on 400 sieves and poured on extraction dish. Eggs were allowed to hatch for 48 hours at 30±2°C in incubator to obtain second stage juveniles (J_2) for inoculation of tomato seedlings.

Multiplication of PGPR

The PGPR (*Pseudomonas fluorescens*, *Bacillus subtilis* and *Azotobacter* spp.) was procured from Department of Plant Protection, SHITS, Allahabad. They were multiplied on nutrient broth. For making the stock solution, their culture was mixed in 100 ml 5% sugar solution to a have the concentration of 10^7 cfu/ml of each PGPR [2].

Mycorrhizal Inoculum

Inoculum was prepared from the fresh root of sorghum stock culture plants infected with *Glomus fasciculatum* (500 spores/g) at a rate of 10g/hole was applied.

The plants were allowed to grow for 60 days and then uprooted to determine the plant growth parameters consisting of height, fresh and dry root and shoot weight and number of galls per root system. Plants were carefully removed from the pots, and the root systems washed free of soil. The root systems were rated for galling on a 0 to 10 scale [4]. The roots were stained with Pheloxin B [27].

Table 1

Treatments	Shoot Length(Cm) 30 Days	Shoot Length(Cm) 60 Days	Fresh Shoot Weight(G)	Dry Shoot Weight(G)	Root Length(Cm)	Fresh Root Weight(G)	Root-Knot Galls Formation/ Plant Root	Number of Juvenile J_2/ Plant Root	Root–Knot Index
Control (plant alone)	15.75	31.25	16.50	4.15	10.25	3.85	0.00	0.00	1
Meloidogyne incognita	8.25	19.25	6.87	1.62	6.00	7.37	48.00	511.25	4
Carbofuran 3G	14.00	28.50	13.85	2.67	9.25	3.70	6.20	45.50	2

Table 1: Contd.,

P. flourescens	13.25	28.00	14.17	3.40	9.00	6.62	25.50	150.75	3
B. subtilis	12.50	27.50	14.02	2.87	9.00	6.95	27.00	179.25	3
Azotobacter spp.	11.50	26.00	13.60	2.65	8.50	7.20	41.50	203.25	4
Mycorrhiza spp.	12.50	27.00	13.87	3.15	9.00	7.10	36.50	187.50	4
P. f.+B. s.	16.00	36.75	17.62	3.75	11.75	4.52	13.25	109.00	3
P. f.+A. spp.	14.50	35.75	16.50	3.57	11.00	5.12	18.50	136.00	3
P. f.+M. spp.	16.75	37.75	15.76	4.00	12.25	4.62	16.00	125.25	3
B. s.+A. spp.	14.25	36.65	16.30	3.57	11.00	5.25	20.5	137.25	3
B. s.+M. spp.	16.25	36.25	16.32	3.80	11.50	4.92	18.00	138.25	3
A. spp.+*M.* spp.	15.75	36.50	15.17	3.80	11.00	5.60	20.75	137.25	3
B.s.+A. spp.+*M.* spp.	18.00	41.75	19.37	5.32	12.50	4.20	12.50	84.50	2.5
P.f.+B. s.+A. spp.	18.50	41.00	21.50	4.65	12.50	3.75	8.25	79.50	2
P.f.+B.s. +M. spp.	19.75	43.50	20.15	6.12	13.50	3.80	6.00	76.75	2
P.f.+A. spp.+*M.* spp.	18.25	43.00	19.02	5.12	13.00	4.08	9.50	81.50	2
P.f.+B. s.+M. spp.+*A.* spp.	18.75	42.25	20.52	5.55	13.25	4.00	8.85	74.00	2
F- test	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**S**
S. Ed.(±)	**0.774**	**1.013**	**1.02**	**0.362**	**0.69**	**0.14**	**1.623**	**9.092**	**0.096**
C. D. (P = 0.05)	**1.626**	**2.128**	**2.143**	**0.761**	**1.45**	**0.293**	**3.408**	**19.094**	**0.202**

RESULTS

Strains of certain rhizobacteria and AMF alone and in combination varied in response to control root-knot nematode. Data presented in Tables. 1 revealed the effect of three isolates of PGPR (*P. fluorescens*, *B. subtilis* and *Azotobacter* spp.) and AMF (*Glomus fasciculatum*) on the plant growth parameters (plant height (cm), shoot fresh and dry weight (g), root length (cm) and root fresh weight (g)) of brinjal and reproduction of nematode (second stage juveniles J_2 and galls/root).

At 60 days after transplanting Significantly highest plant height (cm), dry shoot weight (g) and root length (cm) were found in combined application of *Pseudomonas flourescens, Bacillus subtilis and Glomus fasciculatum*. (43.50cm, 6.12g and 13.50cm, respectively) as compared to control (19.25cm, 1.62g and 6g respectively). Maximum fresh shoot weight (12.50g) was observed in combined application of *P. flourescens, B. subtilis, Azotobacter* sp. as compared to control (6.87g). Mimum fresh root weight (3.70) was observed in (Carbofuran 3G) followed by combined application of *P. flourescens, B. subtilis, Azotobacter* sp. as compared to control (7.37).

Significantly highest decreased in the number of root-knot nematode (6) were found in combined application of *P. flourescens, B. subtilis* and *G. fasciculatum* as compared to control (69).

Maximum decreased in the number of second stage juveniles J_2 (76) were found in combined application of *P. flourescens, B. subtilis, G. fasciculatum* and *Azotobacter* spp. as compared to control (511).

DISCUSSIONS

Combinations of strains of certain rhizobacteria with mycorrhizal fungi showed more effective in both, plant growth parameters and reduce nematode reproduction as compared with individually treatments. Single treatments having (*P. fluorescens, B. subtilis*) and Mycorrhizal fungi showed intermediary effects on plant growth, nematode reproduction

and least effect was found in the treatment having *Azotobacter* spp. The use of plant growth promoting rhizo bacteria (PGPR) promotes plant growth and development through a variety of mechanisms. PGPR have been reported to improve plant growth either through direct stimulation by the synthesis of phytohormones [31] or by decreasing the effect of pathogens [30]. Some rhizobacteria (*Bacillus* spp.) have been found to produce lipo peptides, surfactins, bacillomycin D, and fengycins, which are secondary metabolites mainly with inhabitant pathogen activity [6]. Also some species of *Pseudomonas* bacteria were recorded as highly aggressive colonizers of the rhizosphere of various crop plants and has a broad spectrum antagonistic activity against plant pathogens like nematodes [16, 30]. In addition to some species of *Pseudomonas*, *Bacillus* are reported to induce systemic resistance in plants against invading pathogens and antagonists to root-knot nematodes of *Meloidogyne* spp.[23, 13].

Arbuscular mycorrhizal fungi, improved plant vigor and growth to offset yield loss normally caused by nematodes and physiologically alteration or reduction of root exudates that are responsible for chemotactic attraction of nematodes or directly retarding nematode development or reproduction within the root tissue, enhance and encourage the endophytes and endoparasitic-nematodes to compete for the same site in the root. and higher chitinase activity and ß-1, 3-glucana in roots, as also reported by [10, 21], as well as enhance the host tolerance and augmenting resistance through the increasing of root growth and slowing down nematodes development, changes in root exudates which decrease attraction of nematode and attracted the plant growth promoting bacteria and an increase in phenols in roots, as also reported by [26, 8].

The probable reason for the present findings may be that root knot nematode produces galls in the roots which increase the root weight due to malfunction of the root. The bacteria which effectively decreased the number of galls as well as the fresh root weight were *P. fluorescens*, followed by *B. subtilis*, *G. fasciculatum* and *Azotobacter* spp. The combination treatments proved more effective in decreasing the number of galls, number of second stage juveniles J_2 and root weight (g). While *Azotobacter* spp. was found to have the minimum effect to decrease the number of galls, number of second stage juveniles J_2 and root weight (g) as compared to control. The results are similar to the results of [2]. They reported that the bacteria *P. fluorescens*, *P. putida*, *Bacillus* spp. and *Azotobacter* spp. which effectively decreased females per root system, J_2/one g of root, galls per root system and production of egg masses per root system. And the minimum effect was observed in *Azotobacter* spp. The reduction of galls and number of egg masses by PGPR, as found in our study, agrees with [1, 12, 24, and 14].

CONCLUSIONS

Use of rhizobacteria (*Pseudomonas fluorescens* @ 2ml/kg, *Bacillus subtilis* @ 2ml/kg, *Azotobacter* spp. @ 2ml/kg) and AMF (*Glomus fasciculatum***@** 10g/kg) alone or in combination against *Meloidogyne incognita* (Root-knot disease) on brinjal effectively minimized the incidence of root-knot-galls formation, larval population per root system and increased the plant height (cm), root length (cm), fresh and dry weight (g). The present findings are limited to single trial under Allahabad, Uttar Pradesh, India Agro-climatic conditions as such for validation of the results more trials should be taken up in the future.

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APPENDICES

Figure 1: Source of *Meloidogyne incognita*

Figure 2: View of Experiment in the Net House

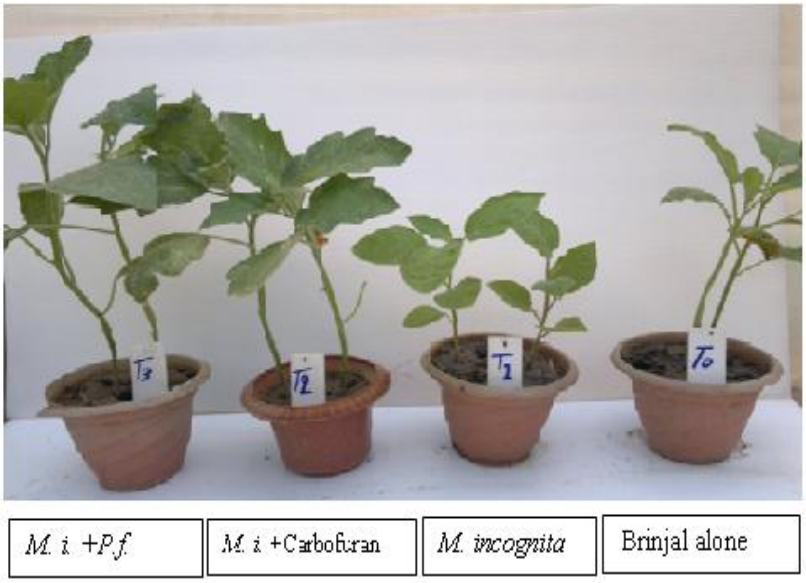

Figure 3: Effect of Treatments on the Growth of Brinjal at 60 Day after Inoculations of *M. incognita*

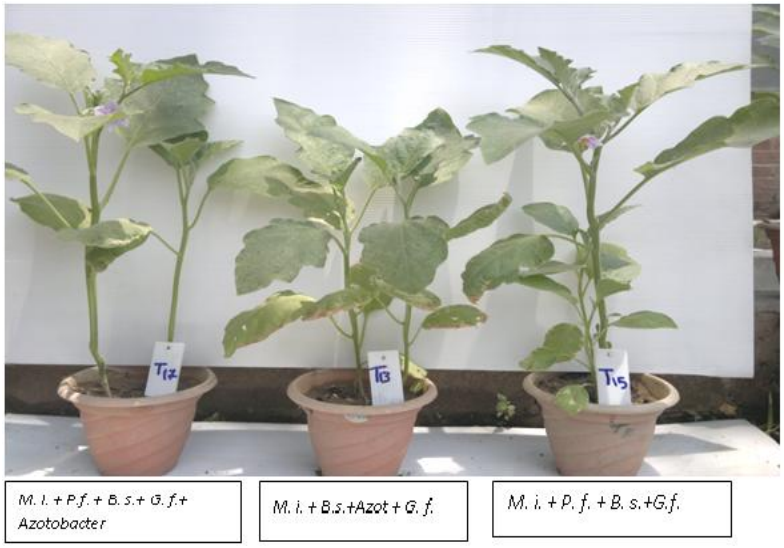

Figure 4a

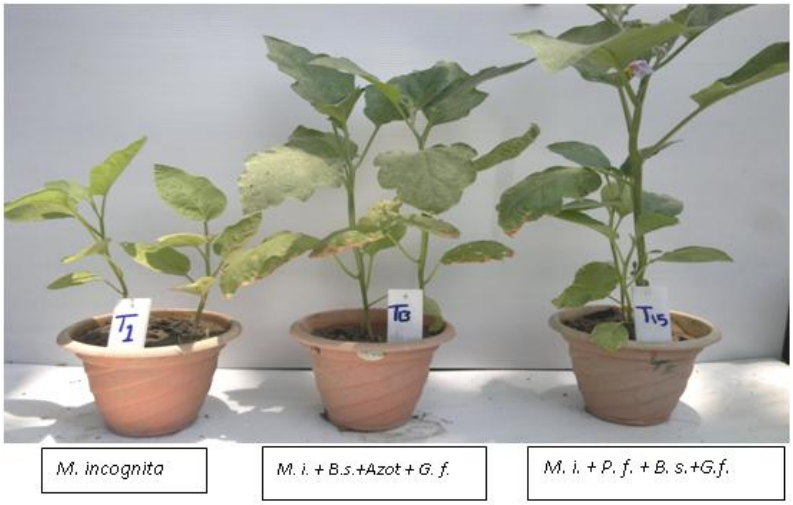

Figure 4b

Figure 4a, b: Response of Bio-Agents against *Meloidogyne incognita* on Brinjal Plants